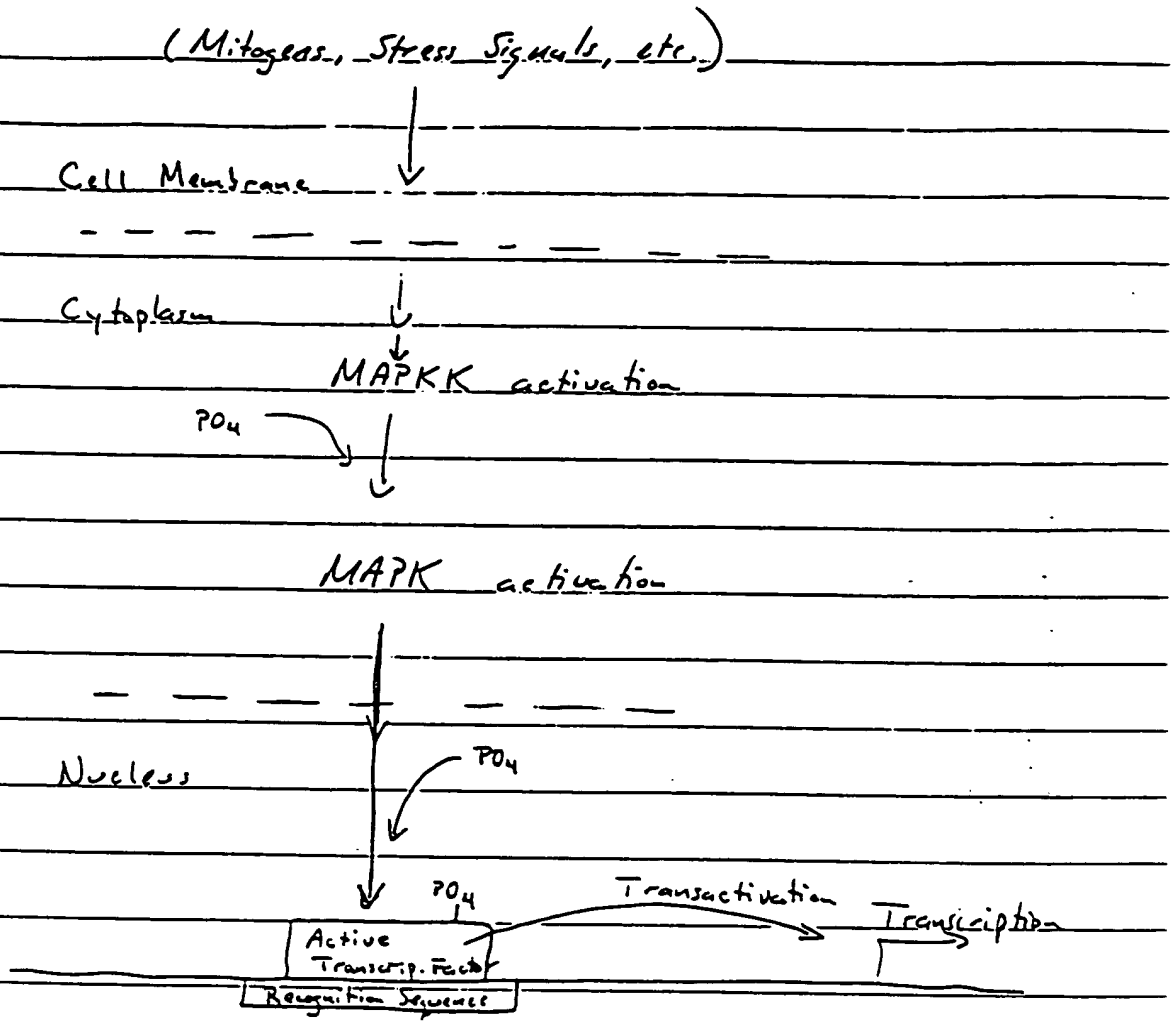
CHOP

Figure


Extracellular Stimuli
(Mitogens, Stress Signals, etc.)
Cell Membrane
Cytoplasm
MAPKK activation
PO4
MAPK activation
Nucleus
PO4
PO4
Transactivation
Transcription
Active Transcrip. Factor
Recognition Sequence

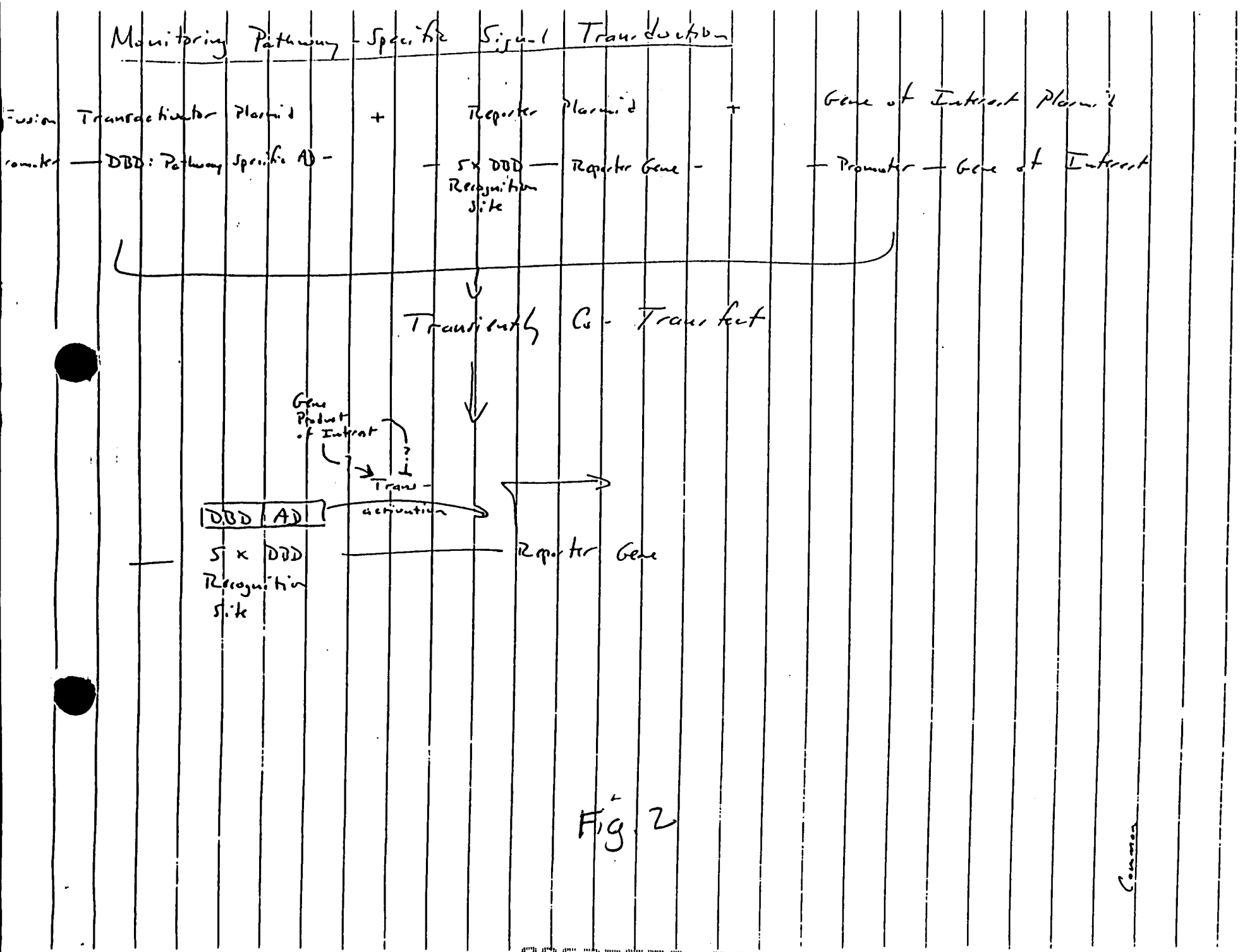


Fig. 2

Figure

Selection of Stable Reporter Cell Lines

Reporter Construct (w/ linked DBD element)

↓

Stably transfect

↓

Screen for clones with low background of Reporter activity and strong response to DBD-bearing activator(s)

↓

"Stable Reporter Cell Line"

↓

Stably transfect with fusion transactivator plasmid

↓

Screen for clones with strong response to pathway-specific upstream activator(s)

↓

"Pathway-Specific Stable Reporter Cell Line"

4.1.1. pFR-Luc Plasmid

Fig. 4

Sequence of GAL4 Binding Element in the pFR-Luc Plasmid

GT CGGAGTACTGTCCTCCG AG CGGAGTACTGTCCTCCG
AG CGGAGTACTGTCCTCCG AG CGGAGTACTGTCCTCCG
AG CGGAGTACTGTCCTCCG AG CGGAGACTCTAGAGGG
TATATAATGGATCCCCGGGT AC CGAGCTCGAATTC--
--CAGCTTGGCATTCCGGTACTGTTGGTAAATG--**Luciferase**

4.1.2. Fusion Transactivator Plasmids

Figure 5

GAL4 (1-147)

c-Jun (1-223)
Elk1 (307-427)
CHOP (1-101)
CREB (1-283)

4.1.3. Control Plasmids

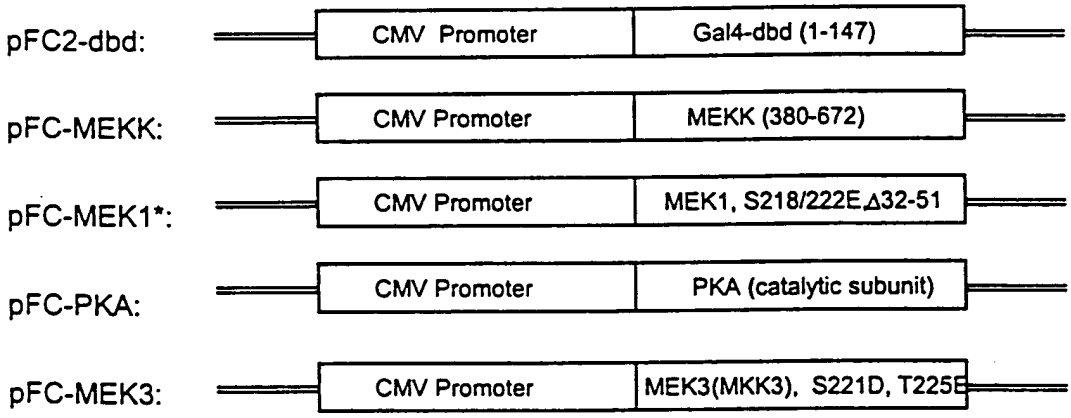


Figure 7

will tailor to each application

4.1.4. pFA-CMV Plasmid

pFA-CMV Plasmid

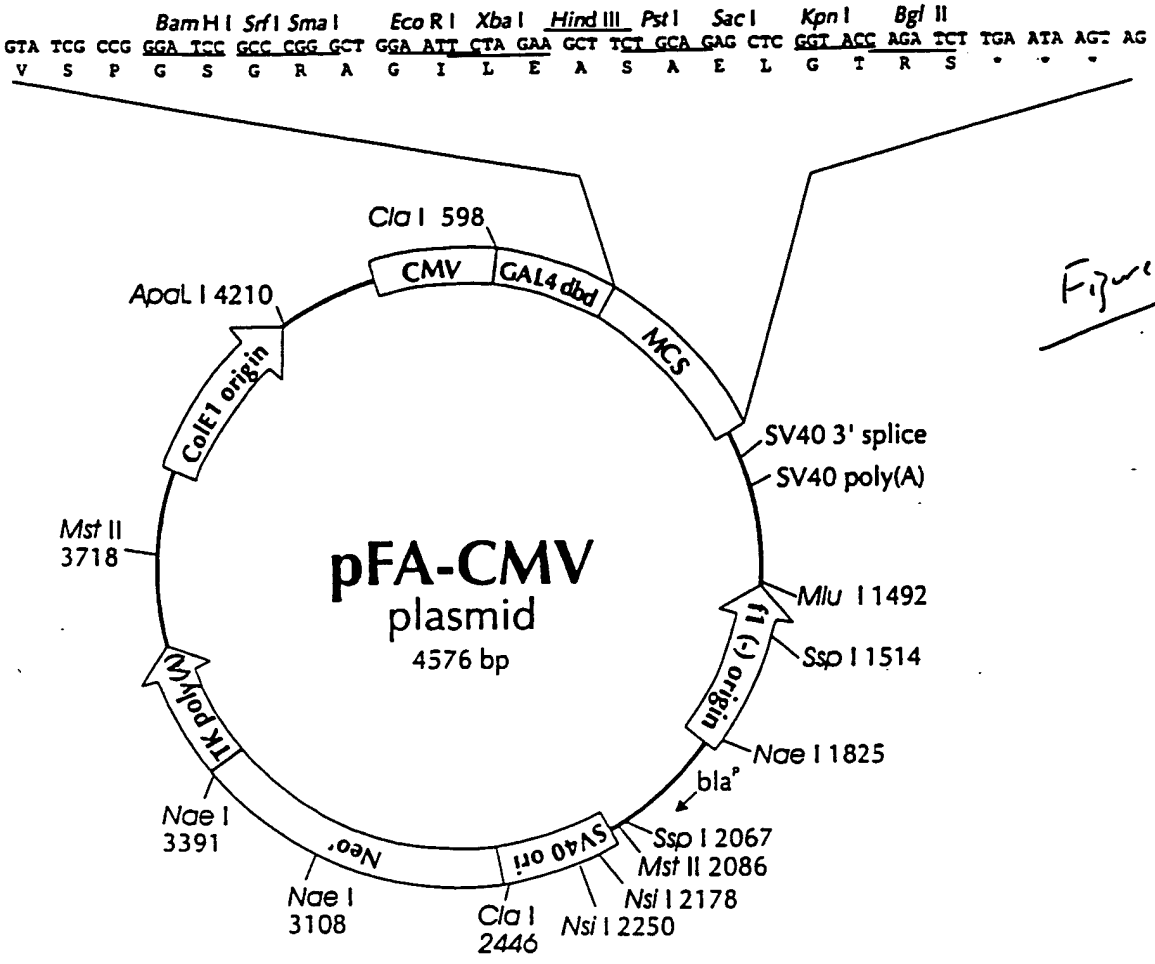


Figure 6

4.2. Preparation of medium and reagents

Luciferase Assay Reagent (1×)
- 40.0 mM tricine (pH 7.8)
- 0.5 mM ATP
- 10 mM $MgSO_4$
- 0.5 mM EDTA
- 10.0 mM DTT
- 0.5 mM coenzyme A
- 0.5 mM luciferin

Cell Lysis Buffer (5×)
- 40 mM tricine (pH 7.8)
- 50 mM NaCl
- 2 mM EDTA
- 1 mM $MgSO_4$
- 5 mM DTT
- 1% Triton® X-100

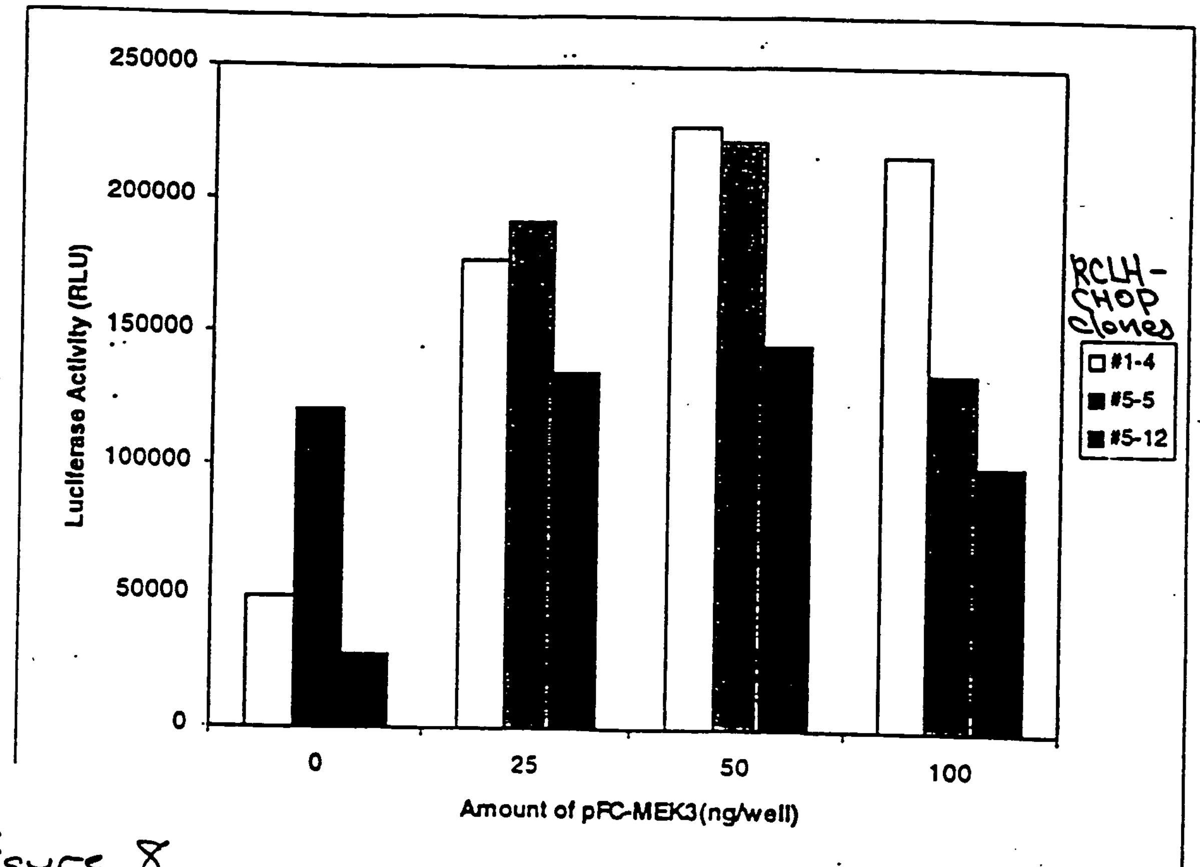

250000
200000
150000
100000
50000
0
Luciferase Activity (RLU)
0
25
50
100
Amount of pFC-MEK3(ng/well)
RCLH-CHOP Clones
#1-4
#5-5
#5-12

Figure 8

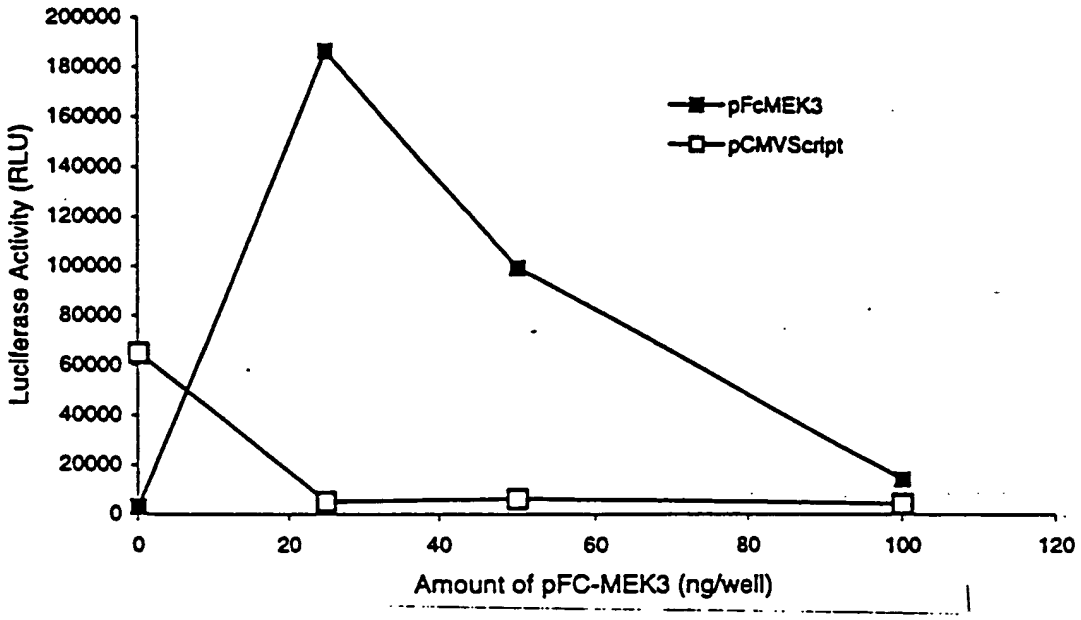


Figure 9

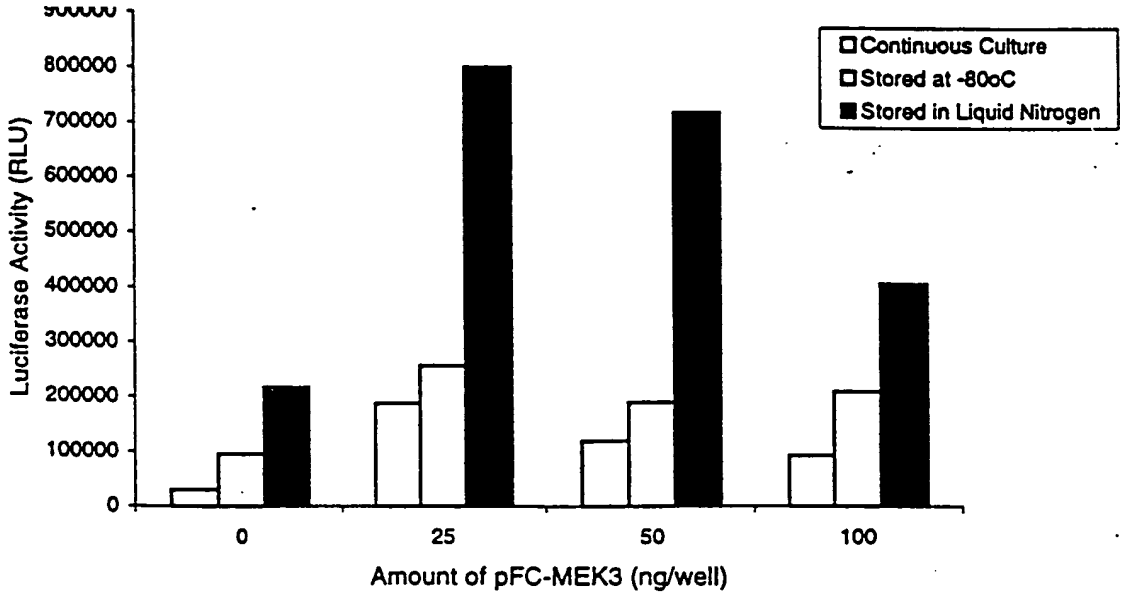


Figure 10